

The natural feed additive caprylic acid decreases *Campylobacter jejuni* colonization in market-aged broiler chickens¹

F. Solis de los Santos,* A. M. Donoghue,† K. Venkitanarayanan,‡ J. H. Metcalf,* I. Reyes-Herrera,* M. L. Dirain,* V. F. Aguiar,* P. J. Blore,* and D. J. Donoghue*²

*Poultry Science Department, University of Arkansas, Fayetteville 72701; †Poultry Production and Product Safety Research Unit, Agricultural Research Service, USDA, Fayetteville, AR 72701; and ‡Department of Animal Science, University of Connecticut, Storrs 06269

ABSTRACT *Campylobacter* causes human foodborne illness, and epidemiological evidence indicates poultry and poultry products as a significant source of human infection. Decreasing *Campylobacter* in the poultry intestinal tract would decrease contamination of poultry products. Caprylic acid is a medium-chain fatty acid reported to be effective in killing a variety of bacterial pathogens, including *Campylobacter jejuni*, but its effect has not been investigated in the control of *C. jejuni* in preslaughter market-aged poultry already colonized with this bacterium. The objective of this study was to determine the therapeutic effect of caprylic acid on *C. jejuni* counts in the cecal contents of 42-d-old chickens. Four trials were conducted. In the first 2 trials, day-of-hatch chicks (n = 60 per trial) were assigned to 6 treatment groups (n = 10 birds per treatment group): positive controls (*Campylobacter*, no caprylic acid), 0.7 or 1.4% of caprylic acid in feed for the last 3 d of the

trial with or without a 12-h feed withdrawal. Treatments were similar for trials 3 and 4 except the doses used were 0.35 or 0.7% caprylic acid supplementation for the last 7 d of the trial. On d 42, ceca were collected and *Campylobacter* counts determined. The supplementation of caprylic acid at 0.35 and 0.7% consistently decreased ($P < 0.05$) the colonization of *C. jejuni* in the chicken ceca compared with positive control treatment. When these treatments were evaluated after a 12-h feed withdrawal period, 0.7% caprylic acid decreased *Campylobacter* colonization in the 3-d treatment supplementation. Body weight and feed consumption did not differ between the caprylic acid and control groups. The results suggest that therapeutic supplementation of caprylic acid in the feed can effectively decrease *Campylobacter* in market-aged chickens and may be a potential treatment for decreasing pathogen carriage in poultry.

Key words: caprylic acid, medium-chain fatty acid, *Campylobacter*, chicken, feed withdrawal period

2009 Poultry Science 88:61–64

doi:10.3382/ps.2008-00228

INTRODUCTION

Campylobacter jejuni is one of the 2 most commonly reported bacterial causes of human foodborne illness in the United States, and epidemiological evidence indicates poultry and poultry products to be a significant source of human *Campylobacter* infection (Oosterom et al., 1984; Friedman et al., 2004; Centers for Disease Control, 2007). Even with biosecurity measures, *Campylobacter* colonization is widespread in most poul-

try flocks (Wallace et al., 1998; Berrang et al., 2000; Loc Carrillo et al., 2005).

Recently, Thormar et al. (2006) reported that monocaprin, the monoglyceride of capric acid (C10:0) was effective in killing significant populations of *C. jejuni* in chicken feed. Caprylic acid is a medium-chain fatty acid with 8 carbons naturally found in breast milk, bovine milk (Jensen, 2002), and coconut oil (Jensen et al., 1990; Sprong et al., 2001). It is a food-grade compound classified as generally recognized as safe by the US Food and Drug Administration. Previously, caprylic acid was found effective in killing a variety of bacterial pathogens, including *Salmonella* Enteritidis in chicken cecal contents (Vasudevan et al., 2005), *Escherichia coli* 0157:H7 in bovine rumen fluid (Annamalai et al., 2004), and *Staphylococcus aureus* in bovine milk (Nair et al., 2005). We recently demonstrated the prophylactic efficacy of feed supplemented with caprylic acid against *C. jejuni* carriage in 10-d-old broiler chicks (Solis de los

©2009 Poultry Science Association Inc.

Received June 4, 2008.

Accepted August 24, 2008.

¹Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that are suitable.

²Corresponding author: ddonogh@uark.edu

Santos, 2008a). In addition, supplementation of caprylic acid in feed for 72 h before necropsy showed therapeutic efficacy against *Campylobacter* in 15-d-old broiler chicks (Solis de los Santos, 2008b). However, the ability of caprylic acid to decrease *Campylobacter* in market-age birds, with or without a feed withdrawal period, has not been investigated. Therefore, the objective of the present study was to determine the therapeutic efficacy of caprylic acid supplementation in broiler chicken finisher feed for 3 or 7 d with and without a 12-h feed withdrawal period on *C. jejuni* populations in the cecal content of market-age broiler chickens.

MATERIALS AND METHODS

Experimental Design

In the first 2 of 4 trials, day-of-hatch chicks ($n = 60$ per trial) were assigned to 6 treatment groups ($n = 10$ birds per treatment group): positive controls (*Campylobacter*, no caprylic acid), 0.7 or 1.4% of caprylic acid in feed for the last 3 d of the trial with or without a 12-h feed withdrawal. Treatments were similar for trials 3 and 4 except the doses used were 0.35 or 0.7% caprylic acid supplementation for the last 7 d of the trial. On day of hatch, chicks were tagged, weighed, and put into floor pens with dimensions of 3.06 m² (33 ft²) equipped with fresh pine litter. Water and feed were provided ad libitum. On d 21 of age, chicks were inoculated with *C. jejuni*, and on 42 d of age, chickens were killed by CO₂ and cecal contents were collected for *Campylobacter* enumeration.

Bacterial Strains, Dosing, and Cecal *Campylobacter* Enumeration

A 5-strain mixture of wild-type *C. jejuni* isolated from chickens was used to colonize the birds as described previously (Farnell et al., 2005). On d 21 of age, seeder chicks ($n = 5$ per treatment pen) were orally challenged with 250 μ L of inoculum using a 1-mL syringe connected to a stainless steel and sterilized cannula. The bacterial population in the inoculum used to challenge the birds in all trials was approximately 2.0×10^6 cfu/mL. Cecal *Campylobacter* content was enumerated by the procedure of Cole et al. (2006). Briefly, ceca from each bird were transferred to a sterile plastic bag, and the contents were squeezed into 15-mL tubes and serially diluted (1:10) with Butterfield's phosphate diluent (6.8% KH₂PO₄; pH 7.2) and inoculated on labeled *Campylobacter* Line agar plates (Line, 2001). The plates were incubated for 48 h at 42°C under microaerophilic conditions. Direct bacterial counts were recorded and converted to colony-forming units per milliliter of the cecal content. Representative colonies were identified as *C. jejuni* by latex agglutination test (Panbio Inc., Columbia, MD) and further confirmed using API-Campy (Biomerieux, Durham, NC).

BW, Feed Consumption, and Cecal Content pH Determination

Birds from each treatment group were weighed on d 42, and the feed consumption during the dosing period was determined by weighing feed before and immediately after the 3- or 7-d dosing period. For cecal pH determination, cecal contents from each bird were squeezed into 10-mL tubes, diluted (1:9) with distilled water, vortexed, and read using a glass pH meter (Hume et al., 1993).

Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 2002). The numbers of *Campylobacter* colonies were logarithmically transformed (\log_{10} cfu/g) before analysis to achieve homogeneity of variance (Byrd et al., 2003). Treatment means were partitioned by LSMEANS analysis (SAS Institute, 2002). A probability of $P < 0.05$ was required for statistical significance. The data in Figure 1 are shown as arithmetic means for clarity of presentation.

RESULTS

Both the 3- and 7-d supplementation with 0.7% caprylic acid consistently decreased cecal *C. jejuni* counts by approximately 3 and 2 logs cfu/g in trials 1 and 2, respectively, and by 3 logs cfu/g in trials 3 and 4, compared with the positive control treatment group (Figure 1). Use of the 1.4% dose resulted in an inconsistent effect. In trial 1, this dose of caprylic acid decreased cecal *Campylobacter* counts compared with positive controls but was not effective in trial 2. The 0.35% dose, however, was effective in both trials when fed for 7 d (Figure 1). When the effect of a 12-h feed withdrawal was evaluated, the 0.7% dose fed for 3 d decreased *Campylobacter* counts by approximately 3 logs cfu/g when compared with positive controls for trials 1 and 2 (4.8 ± 1.1 vs. 7.4 ± 0.4 and 3.9 ± 1.1 vs. 7.1 ± 0.5 cfu/g for the 0.7% caprylic acid dose vs. controls, trials 1 and 2, respectively). For the 7-d dosing period, however, the 0.7% dose did not decrease *Campylobacter* counts in either trial (7.8 ± 0.2 vs. 7.3 ± 0.5 and 6.9 ± 0.3 vs. 7.8 ± 0.2 for the 0.7% caprylic acid dose vs. controls, trials 3 and 4, respectively). Both the 1.4% dose for 3 d and the 0.35% dose for 7 d with a 12-h feed withdrawal produced inconsistent reductions in cecal *Campylobacter* counts (4.1 ± 1.0 vs. 7.4 ± 0.4 and 6.9 ± 0.3 vs. 7.1 ± 0.5 for the 1.4% dose vs. controls, trials 1 and 2, respectively; 7.0 ± 0.4 vs. 7.3 ± 0.5 or 4.4 ± 0.7 vs. 7.8 ± 0.2 for the 0.35% dose vs. controls, trials 3 and 4, respectively).

Body weights and feed consumption (data not shown) were not affected ($P > 0.05$) by the supplementation of caprylic acid treatments in feed compared with positive control treatments in any of the trials. Cecal content pH was either not different ($P > 0.05$) or not consistently different between replicate trials (data not shown).

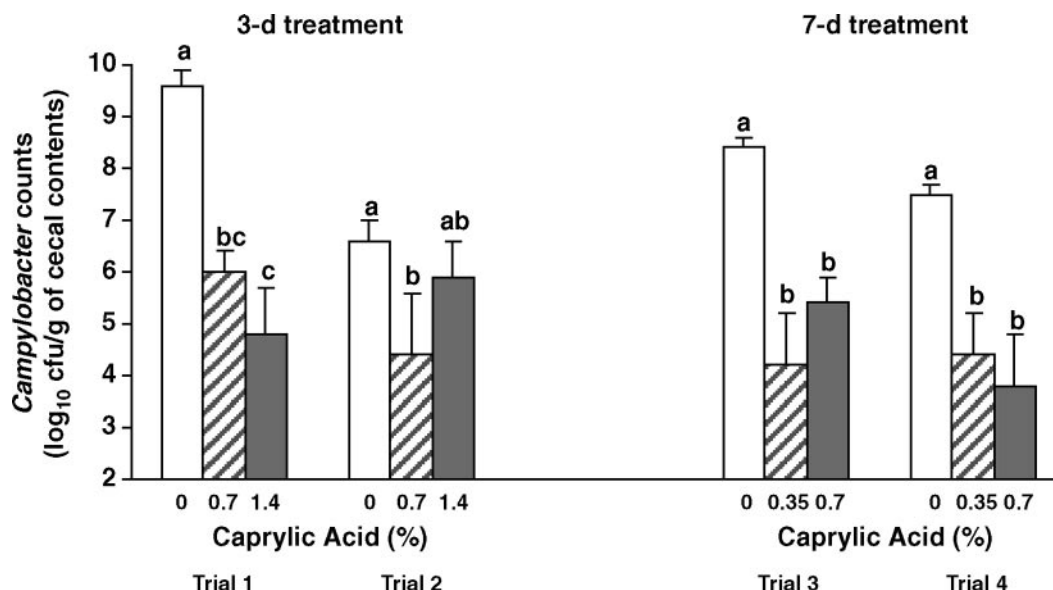


Figure 1. Cecal *Campylobacter jejuni* counts in 42-d-old broiler chickens ($n = 10$ birds/treatment per trial) fed caprylic acid 3 d (trial 1 and 2) or 7 d (trial 3 and 4) before necropsy. ^{a-c}Columns with no common letters differ significantly ($P < 0.05$).

DISCUSSION

The results of this study demonstrate that caprylic acid supplemented at 0.7% for 3 or 7 d in the feed consistently decreased cecal *C. jejuni* counts when compared with positive controls in market-age chickens. When a 12-h feed withdrawal period was evaluated, the 0.7% dose also decreased *C. jejuni* counts substantially when supplemented for 3 d, but not for 7 d. These results suggest that the 0.7% dose for 3 d, with or without feed withdrawal, is the most efficacious treatment for decreasing enteric *Campylobacter* counts in market-aged broilers. These results are consistent with previous studies in which prophylactic efficacy (Solis de los Santos et al., 2008a) and therapeutic efficacy of feed-supplemented caprylic acid against *Campylobacter* was observed in younger chicks (Solis de los Santos et al., 2008b).

The ability of caprylic acid to decrease enteric *Campylobacter* counts after feed withdrawal is an important consideration. Feed removal before slaughter is a common industry practice to decrease intestinal content and intestinal rupture, thereby decreasing the probability of carcass contamination (Papa and Dickens, 1989; Northcutt et al., 1997; Thompson and Applegate, 2006). Unfortunately, feed withdrawal can be associated with increased pecking of manure-contaminated litter, which may increase the amount of pathogens in the intestine of the chickens (Ramirez et al., 1997; Byrd et al., 1998; Corrier et al., 1999; Thompson and Applegate, 2006). Thus, dosing with 0.7% caprylic acid in the feed for 3 d allows the use of a 12-h feed withdrawal period in addition to decreasing cecal *Campylobacter* populations.

Caprylic acid is a natural and relatively inexpensive compound; its supplementation through feed represents a practical and economical strategy for poultry farmers for decreasing *C. jejuni* carriage in chickens. In the pres-

ent study, the 0.7% dose of caprylic acid therapeutically decreased cecal *C. jejuni* counts of market-aged broiler chickens by approximately 3 logs. This could have significant beneficial implications on food safety, because, during processing, enteric contents can contaminate the carcass, thereby resulting in foodborne transmission of *C. jejuni* (Rosenquist et al., 2006; Allen et al., 2007). It has been estimated that a 2-log reduction in *C. jejuni* populations on poultry carcass contaminations could bring about a 30-fold reduction in human campylobacteriosis cases (Rosenquist et al., 2003). Furthermore, this treatment is consistent with our previous studies and did not have any effect on BW or feed consumption (Solis de los Santos et al., 2008a,b).

It is unclear how caprylic acid decreases enteric *Campylobacter* counts in chickens, but it may be due to either an indirect effect via alterations in the enteric microflora or environment or a direct antimicrobial effect. Previous research from our laboratory demonstrated a physical alteration of the gastrointestinal tract associated with a reduction in *Campylobacter* content after bacteriocin treatment (Cole et al., 2006). Dosing with caprylic acid, however, did not produce similar effects (Solis de los Santos et al., 2008b). It would also appear that a decrease in intestinal pH is not responsible for caprylic acid-mediated *C. jejuni* reduction, because its treatment had limited, if any, effect on cecal pH in the current study. It is also possible that caprylic acid changes the intestinal microflora, similar to a pre- or probiotic, decreasing enteric *Campylobacter* content. Studies are currently underway to investigate this possibility. Caprylic acid may also directly inhibit the expression of virulence factors necessary for *C. jejuni* colonization in chicks. For example, medium-chain fatty acids have been found to significantly decrease the invasiveness of *Salmonella* Enteritidis in invasion assays by decreasing the expression of *hilA*, a regulator of *Sal-*

monella virulence genes, which are directly involved in the invasion of intestinal epithelial cells (Van Immerseel et al., 2004). However, additional research is necessary to confirm these hypotheses. In conclusion, the results of the present study indicate that the use of select doses of caprylic acid in feed before slaughter decreases cecal *Campylobacter* counts in market-age chickens and may provide a strategy for decreasing chicken-related outbreaks of this foodborne pathogen.

ACKNOWLEDGMENTS

This work was supported in part by USDA, Cooperative State Research, Education, and Extension Service National Integrated Food Safety Program 2006-02429 to K. Venkitanarayanan and D. J. Donoghue.

REFERENCES

- Allen, V. M., S. A. Bull, J. E. Corry, G. Domingue, F. Jørgensen, J. A. Frost, R. Whyte, A. Gonzalez, N. Elviss, and T. J. Humphrey. 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonization. *Int. J. Food Microbiol.* 113:54–61.
- Annamalai, T., M. K. Mohan Nair, P. Marek, P. Vasudevan, D. Schreiber, R. Knight, T. Hoagland, and K. Venkitanarayanan. 2004. In vitro inactivation of *Escherichia coli* O157:H7 in bovine rumen fluid by caprylic acid. *J. Food Prot.* 67:884–888.
- Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcasses prior to scalding. *Poult. Sci.* 79:286–290.
- Byrd, J. A., R. C. Anderson, T. R. Callaway, R. W. Moore, K. D. Knappe, L. F. Kubena, R. L. Ziprin, and D. J. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. *Poult. Sci.* 82:1403–1406.
- Byrd, J. A., D. E. Corrier, M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Incidence of *Campylobacter* in crops of preharvest market-age broiler chickens. *Poult. Sci.* 77:1303–1305.
- Centers for Disease Control. 2007. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2006. *Morb. Mortal. Wkly. Rep.* 56:336–339.
- Cole, K., M. B. Farnell, A. M. Donoghue, N. J. Stern, E. A. Svetoch, B. N. Eruslanov, L. I. Volodina, Y. N. Kovalev, V. V. Perelygin, E. V. Mitsevich, I. P. Mitsevich, V. P. Levchuk, V. D. Pokhilenko, V. N. Borzenkov, O. E. Svetoch, T. Y. Kudryavtseva, I. Reyes-Herrera, P. J. Blore, F. Solis de los Santos, and D. J. Donoghue. 2006. Bacteriocins reduce *Campylobacter* colonization and alter gut morphology in turkey poults. *Poult. Sci.* 85:1570–1575.
- Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. *Poult. Sci.* 78:45–49.
- Farnell, M. B., A. M. Donoghue, K. Cole, I. Reyes-Herrera, P. J. Blore, and D. J. Donoghue. 2005. *Campylobacter* susceptibility to ciprofloxacin and corresponding fluoroquinolone concentrations within the gastrointestinal tracts of chickens. *J. Appl. Microbiol.* 99:1043–1059.
- Friedman, C. R., R. M. Hoekstra, M. Samuel, R. Marcus, J. Bender, B. Shiferaw, S. Reddy, S. D. Ahuja, D. L. Helfrick, F. Hardnett, M. Carter, B. Anderson, and R. V. Tauxe. 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. *Clin. Infect. Dis.* 38:S285–S296.
- Hume, M., D. E. Corrier, S. Ambrus, A. Hinton Jr., and J. R. DeLoach. 1993. Effectiveness of dietary propionic acid in controlling *Salmonella* Typhimurium colonization in broiler chicks. *Avian Dis.* 37:1051–1056.
- Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* 85:295–350.
- Jensen, R. G., A. M. Ferris, C. J. Lammi-Keefe, and R. A. Henderson. 1990. Lipids of bovine and human milks: A comparison. *J. Dairy Sci.* 73:223–240.
- Line, J. E. 2001. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. *J. Food Prot.* 64:1711–1715.
- Loc Carrillo, C., R. J. Atterbury, A. El-Shibiny, P. L. Connerton, E. Dillon, A. Scott, and I. F. Connerton. 2005. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Appl. Environ. Microbiol.* 71:6554–6563.
- Nair, M. K., J. Joy, P. Vasudevan, L. Hinckley, T. A. Hoagland, and K. S. Venkitanarayanan. 2005. Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. *J. Dairy Sci.* 88:3488–3495.
- Northcutt, J. K., S. I. Savage, and L. R. Vest. 1997. Relationship between feed withdrawal and viscera condition. *Poult. Sci.* 76:410–414.
- Oosterom, J., C. H. den Uyl, J. R. J. Banffer, and J. Huisman. 1984. Epidemiological investigations on *Campylobacter jejuni* in households with primary infection. *J. Hyg. (Camb.)* 92:325–332.
- Papa, C. M., and J. A. Dickens. 1989. Lower gut contents and defecatory responses of broiler chickens as affected by feed withdrawal and electrical treatment at slaughter. *Poult. Sci.* 68:1478–1484.
- Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak Jr., M. E. Hume, D. E. Corrier, J. R. DeLoach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. *Poult. Sci.* 76:654–656.
- Rosenquist, H., N. L. Nielsen, H. M. Sommer, B. Norrung, and B. B. Christensen. 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *Int. J. Food Microbiol.* 83:87–103.
- Rosenquist, H., H. M. Sommer, N. L. Nielsen, and B. B. Christensen. 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *Int. J. Food Microbiol.* 108:226–232.
- SAS Institute. 2002. SAS/STAT® User's Guide: Release 9.03 Edition. SAS Institute Inc., Cary, NC.
- Solis de los Santos, F., A. M. Donoghue, K. Venkitanarayanan, M. L. Dirain, I. Reyes-Herrera, P. J. Blore, and D. J. Donoghue. 2008a. Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in young broiler chickens. *Poult. Sci.* 87:800–804.
- Solis de los Santos, F., A. M. Donoghue, K. Venkitanarayanan, I. Reyes-Herrera, J. H. Metcalf, M. L. Dirain, V. F. Aguiar, P. J. Blore, and D. J. Donoghue. 2008b. Therapeutic supplementation of caprylic acid in feed reduces *Campylobacter jejuni* in broiler chicks. *Appl. Environ. Microbiol.* 74:4564–4566.
- Sprong, R. C., M. F. Hulstine, and R. van der Meer. 2001. Bactericidal activities of milk lipids. *Antimicrob. Agents Chemother.* 45:1298–1301.
- Thompson, K. L., and T. J. Applegate. 2006. Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poult. Sci.* 85:1535–1540.
- Thormar, H., H. Hilmarsson, and G. Bergsson. 2006. Stable concentrated emulsions of the 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the food-borne bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. *Appl. Environ. Microbiol.* 72:522–526.
- Van Immerseel, F., J. De Buck, F. Boyen, L. Bohez, F. Pasmans, J. Volf, M. Sevcik, I. Rychlik, F. Haesebrouck, and R. Ducatelle. 2004. Medium-chain fatty acids decrease colonization and invasion through *hilA* suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. *Appl. Environ. Microbiol.* 70:3582–3587.
- Vasudevan, P., M. Patrick, M. Nair, T. Annamalai, M. Darre, M. Khan, and K. Venkitanarayanan. 2005. In vitro inactivation of *Salmonella* Enteritidis in chicken cecal contents by caprylic acid. *J. Appl. Poult. Res.* 14:122–125.
- Wallace, J. S., K. N. Stanley, and K. Jones. 1998. The colonization of turkeys by thermophilic campylobacters. *J. Appl. Microbiol.* 85:224–230.